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RESEARCH ARTICLE

# DEVELOPMENT OF MUCOADHESIVE MICROSPHERES OF LEVOFLOXACIN FOR THE TREATMENT OF *H. PYLORI* INFECTION

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## ABSTRACT

The rationale of the present investigation is to develop a new oral drug delivery system utilizing both the concepts of controlled release and mucoadhesiveness, which could remain in stomach and control the drug release for longer period of time and thus to improve the bioavailability of the drug and reducing its dose related side effects. Gelatin/Acrypol 934P mucoadhesive microspheres of Levofloxacin were prepared by Emulsification cross- linking method. Drug and excipients interactions are tested using Fourier Transform-Infrared Spectroscopy indicated no interactions. The average particle size for optimized formulations of Gelatin/Acrypol 934P microspheres were found to be 41.5µm. Photomicrographs revealed that the microspheres were spherical in shape. The drug entrapment efficiency for Gelatin/ Acrypol 934P microspheres was found to be 85.43%. *In vitro* drug release from Gelatin/ Acrypol microspheres showed more than 75% of the drug was released within 8 Hr, while pure drug showed complete drug release within 3 hours. This suggested controlled delivery of Levofloxacin for a longer period. Regression analysis revealed that the drug release from the microspheres was followed zero order kinetics. SEM images suggested spherical shape with smooth surface of microspheres formulations. Optimized formulation of Gelatin/ Acrypol 934P microspheres showed excellent mucoadhesivity i.e., 86.5%. Thus, the proposed Gelatin/ Acrypol 934P mucoadhesive microspheres might make a contribution in complete eradication of *Helicobacter pylori* owing to prolonged stomach residence time and small particle size.

**Key words**: Levofloxacin, *H. pylori*, Gelatin, Acrypol 934P, mucoadhesion, controlled release.

## INTRODUCTION

*H. pylori* is a helix-shaped gram negative, microaerophilic (i.e., it requires oxygen) bacterium, mostly found in the stomach. It is one of the causes for gastritis, gastric ulcers, duodenal ulcers. [1] *H. pylori* used its flagella to burrow into the mucus lining of the stomach to reach the epithelial cells underneath where pH is more neutral. *H. pylori* is able to sense the pH gradient in the mucus and move towards the less acidic region (chemotaxins). The organisms *H. pylori* exclusively reside on the luminal surface of the gastric mucus under the mucus gut layer. [2] Therefore it becomes necessary to develop a drug delivery system, which can protect the drug from the gastric environment. The delivery system should be bioadhesive with a small particle size and at the same time it should target the drug to the bacterial cell lines. An important factor for bioadhesion is the particle size of the drug delivery sysem. Bioadhesive properties were optimized by a reduction of the size of the microparticulate and these improvements were attributed to several factors such as an increase of the adhesive forces, or a prolongation of the GI transit time leading to a higher bioavailability of drugs.[3]

Gastroretentive drug delivery system like mucoadhesive microspheres system would improve the therapeutic effect of antimicrobial drugs. Microspheres are the carrier linked drug delivery system and are small spherical particles with diameter ranges from 1 µm to 1000 µm. Microspheres constitute an important part of novel drug delivery system by virtue of their small size and efficient carrier capacity. Due to their short residence time, bioadhesive characteristics can be coupled to microspheres to develop mucoadhesive microspheres. Drug action can be improved by developing new drug delivery system, such as the mucoadhesive microsphere drug delivery system. These systems remain in close contact with the absorption tissue, the mucous membrane, releasing the drug at the action site leading to a bioavailability increase and both local and systemic effects. The oral route of drug administration constitutes the most convenient and preferred means of drug delivery to systemic circulation

 

of body. Mucoadhesive drug carriers may prolong the residence time in the gastrointestinal tract (GI) because they can adhere to the mucus surface, resulting in an effective localized drug concentration. [4]

Mucoadhesive drug carriers may prolong the residence time in the gastrointestinal tract because they can adhere to the mucus surface, resulting in an effective localized drug concentration. Among several mucoadhesive polymers, Acrypol usually has strong mucoadhesive properties and is known to be biocompatible and nontoxic.[4,5]

## MATERIALS AND METHODS

### Materials

Levofloxacin was a gift sample from SK Pharma, Sagar. Acrypol was a gift sample from Corel Pharma chem., Ahmedabad. Gelatin, Sun flower oil, Acetone, Diethyl ether, Glutaraldehyde, Concentrated Hydrochloric acid, Methanol were purchased from Sd fine chem. Limited, Mumbai.

### Analytical Methods

Absorption spectroscopy is one of the most valuable analytical techniques that is extensively used for qualitative and quantitative analysis of drugs. Although it has certain limitations, it has various advantages such as speed, simplicity, specificity and sensitivity. U.V. spectrophotometry was used for the analysis of Levofloxacin. An easy and simple spectrophotometric method is reported in literature which gives $\lambda_{max}$ for Levofloxacin 293 nm.[6]

### UV Spectrophotometric Method

#### Drug scaning

Pure 10mg of Levofloxacin was dissolved in 100ml of 0.1N Hydrochloric acid. 0.8ml of this solution is further diluted to 10ml with same solvent to obtain 8µg/ml Levofloxacin solution. Scanned spectroscopically in the wavelength region 200 to 400nm. The determined wave length of maximum absorption was at 293nm.

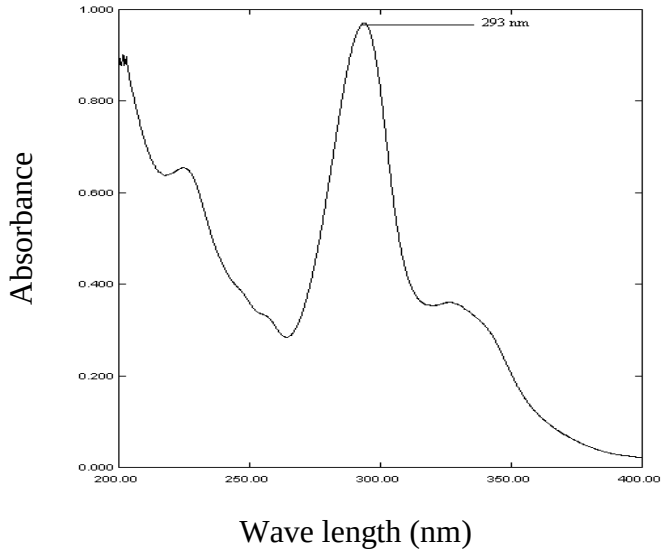


**Figure 1: Scan for Levofloxacin solution (8µg/ml) in 0.1N Hydrochloric acid**

#### Calibration curve of Levofloxacin in 0.1N Hydrochloric acid solution [6]

**Stock solution:** Pure Levofloxacin 50mg was dissolved in 100ml of 0.1N Hydrochloric acid. 5ml of this solution was further diluted to 100ml with same solvent to obtain 25µg/ml.

**Working solution:** From the stock solution (25µg/ml) suitable working solution of different concentrations of 2, 4, 6, 8 & 10µg/ml were made. The absorbance of these dilutions was measured at 293nm. The standard graph of concentration versus absorbance was then plotted. Plot is shown in Figure 1. Each point is an average of three determinations. Slope, y-axis intercept, and regression coefficients were calculated. Table 1 shows the data for Standard plot of Levofloxacin in 0.1N hydrochloric acid solution.

### Characterization of Levofloxacin

#### Melting point [7]

The melting point of the Levofloxacin was determined using capillary tubes. One end of the capillary tube was sealed. The sample was filled and placed in the melting point apparatus. The melting point was noted.

#### FT-IR studies [7]

Levofloxacin was added to the powdered potassium bromide in the ratio of 1:100. The mixture was compacted under pressure (10 tons/cm$^2$) in vacuum to form a transparent pellet. The spectra were obtained by placing the pellet in the IR chamber and the peak intensities were taken.

 

### Drug - Excipient Compatibiliy Studies By FT- IR Studies[7]

The study of drug- excipient compatibility is an important stage in the development of a dosage form as their incompatibiliy can alter the stability and/or the bioavailability of drugs thereby, affecting its safety and/or efficacy. Drug- excipient compatibility was performed by FTIR analysis.

FTIR spectra of physical mixture Levofloxacin (drug) and Gelatin+ Acrypol 934P (excipients) (1:1) ratio recorded on Shimadzu FTIR Spectrophotometer. Sample was added to the powdered potassium bromide in the ratio of 1:100. The mixture was compacted under pressure (10 tons/cm$^2$) in vacuum to form a transparent pellet. The spectra were obtained by placing the pellet in the IR chamber and the peak intensities were taken. The characteristic bands of mixture with excipients were compared with pure drug.

## Formulation and Development[8]

### Preparation of Gelatin/ Acrypol 934P mucoadhesive microspheres by emulsification cross linking method

An aqueous 15ml of 20% aqueous Gelatin solution was prepared by heating at 55- 60˚C. 1000mg of drug was added to the phase. The aqueous phase was emulsified with 100ml of sunflower oil, previously heated to 55- 60$^o$C and the mixture was stirred at 1000 RPM for 5min using mechanical stirrer. To this emulsion, Acrypol 934P (different % with respect to gelatin mass) was added and stirring was prolonged for further 5min. The cross- linker Glutaraldehyde was added to the emulsion. The prepared Gelatin droplets were solidified by a fast cooling process under stirring for 30min. Dehydration was carried out by adding 75ml of acetone under stirring for 5min. The microspheres were separated by filtration and washed 3 times with 50ml of Diethyl ether.

The formulation variables in the preparation of Gelatin/ Acrypol 934P microspheres are tabulated in the Table 1. In the composition of Gelatin/ Acrypol 934P microspheres, Acrypol 934P concentration and stirring speed was varied and remaining formulation variables like Gelatin solution, sun flower oil, Glutaraldehyde, Acetone, Diethyl ether and drug amount were kept constant.

**Table 1: Composition of Gelatin/ Acrypol 934P microspheres formulation to study the effect of Acrypol 934P concentration & stirring speed**

| Formulation variables | GA1 | GA2 | GA3 | GA4 | GA5 |
|---|---|---|---|---|---|
| Gelatin solution %(w/w) | 20% | 20% | 20% | **20%** | 20% |
| Sun flower oil (ml) | 100ml | 100ml | 100ml | **100ml** | 100ml |
| Acrypol 934P (%) | 0.5% | 1% | 2% | **1%** | 1% |
| Speed (RPM) | 500RPM | 500 RPM | 500 RPM | **1000 RPM** | 1500 RPM |
| Gluteraldehyde (%) | 1% | 1% | 1% | **1%** | 1% |
| Acetone (ml) | 75ml | 75ml | 75ml | **75ml** | 75ml |
| Diethyl ether (ml) | 50ml | 50ml | 50ml | **50ml** | 50ml |
| Drug (mg) | 1000mg | 1000mg | 1000mg | **1000mg** | 1000mg |

### Optimization of process variables of prepared system

A preparation Gelatin / Acrypol 934P microsphere involves various process variables, out of which the following variables were selected:

(a) Effect of Acrypol 934P concentration.
(b) Effect of Gluteraldehyde concentration.
(c) Effect of stirring speed.

Effects of these variables were observed on final particle size distribution of microspheres.

## Evaluation of Mucoadhesive Microspheres

### Shape and surface morphology [9]

#### Optical photomicrograph

Microspheres suspension was mounted on glass slide and observed under the optical microscope for their shape. The photomicrographs of both the system are shown in the Figure 7.

#### SEM analysis

The surface morphology was visualized by Scanning Electron Microscopy (SEM). The samples for SEM were prepared by lightly sprinkling the microsphere powder on a double adhesive tape, which stuck to an aluminium stub. The stubs were then coated with gold a thickness of about 300A˚ using a sputter coater. The samples were then randomly scanned and photographs were taken. SEM photographs of the microspheres system is shown in Figure 8 SEM analysis was done using HITACHI S-3700N SEM analysis instrument. It was done to study surface morphology (shape) of microspheres.

### Particle size analysis [19]

Particle size was studied using optical microscope, consisting of eye piece and stage micrometer. Calibration factor was calculated then microspheres were placed on the slide using Liquid Paraffin and size was observed. The particle diameters of 100 microspheres were measured randomly by optical microscope.

 

Average particle size = Σnd/Σn

Here ∑nd = sum of product of frequency & diameter

∑n = total no. of spheres counted

### Percentage yield [10]

Microspheres dried at room temperature were weighed and the yield of microspheres preparation was calculated by using formula:

Percentage yield = [Practical Yield / Theoretical Yield] x 100

### Drug entrapment efficiency [11]

200 mg of accurately weighed microspheres were crushed in a glass mortar and the powdered microspheres were suspended in 10 ml of 0.1 N Hydrochloric acid (pH = 1.2). After 24h, the solution was filtered and the filtrate was analysed for the drug content. The drug entrapment efficiency was calculated using the following formula:

$$\%\text{Entrapment efficiency} = \frac{\text{Amount of drug actually present}}{\text{Theoretical drug load expected}} \times 100$$

### *In- vitro* wash off test for mucoadhesion [12]

The mucoadhesive property of microspheres was evaluated by *in- vitro* wash off test for mucoadhesion. Pieces of goat stomach mucosa were mounted onto glass slides using thread. 100mg of microspheres were spread onto each wet rinse tissue specimen. Immediately thereafter the support was hung onto the arm of USP Disintegration test machine. By operating the disintegrating test machine, the tissue specimen was given a regular up and down movement in 0.1N Hydrochloric acid at 37˚C. At the end of one hour the machine was stopped and the microspheres in the 0.1N Hydrochloric acid was centrifused, dried and weighed. The mucoadhesivity of these microspheres was calculated by the following formula:

$$\text{Percentage Mucoadhesivity} = \frac{\text{Wt. of adhered microspheres}}{\text{Wt. of applied microspheres}} \times 100$$

### *In- vitro* drug release studies[12]

The drug release study was performed using USP paddle apparatus at 37°C±0.5°C and at 100 RPM using 900 ml of 0.1N Hydrochloric acid as a dissolution medium. 300mg of microspheres containing 100mg of pure drug were filled in "0" size hard gelatin capsules were placed in dissolution medium. Perfect sink conditions prevailed during the drug dissolution tests. 5 ml of sample solution was withdrawn at predetermined time intervals and the absorbance of the sample was recorded using UV spectrophotomerically at 293nm. The same experiment was conducted for pure drug Levofloxacin (100 mg).

### Drug Release Kinetics[13]

Drug released from the dosage forms follow the different kinetics rules. The release of the drug from the different dosage forms depends on the various factors like concentration, temperature, light and also the rotating speed of the paddle. There are several mathematical models of release kinetics. Some are described below.

#### Zero-order model

Drug dissolution from dosage forms that do not disaggregate and release the drug slowly can be represented by the equation:

$$\mathbf{Q_0 - Q_t = K_0 t}$$

Rearrangement

$$\mathbf{Qt = Q_0 + K\,t}$$

Where, Qt is the amount of drug dissolved in time *t*. $Q_0$ is the initial amount of drug in the solution (most times, $Q_0$=0) and $K_0$ is the zero order release constant expressed in units of concentration/time.

To study the release kinetics, data obtained from *in vitro* drug release studies were plotted as cumulative amount of drug released *versus* time.

Application: This relationship can be used to describe the drug dissolution of several types of modified release pharmaceutical dosage forms, as in the case of some transdermal systems, as well as matrix tablets with low soluble drugs in coated forms, osmotic systems, etc.

#### First order model

This model has also been used to describe absorption and/or elimination of some drugs, although it is difficult to conceptualize this mechanism on a theoretical basis. The release of the drug which followed first order kinetics can be expressed by the equation**:**

$$\mathbf{dc/\,dt = -kc}$$

Where k is the first order rate constant expressed in units of time $^{-1.}$

This equation can be expressed as:

$$\mathbf{\log C = \log C_0 - Kt / 2.303}$$

Where, $C_0$ is the initial concentration of drug, k is the first order rate constant and t is the time.

The data obtained are plotted as log cumulative percentage of drug remaining *verses* time which would yield a straight line with a slope of –K/2.303.

Application: this relationship can be used to describe the drug dissolution in pharmaceutical dosage forms such as those containing water-soluble drugs in porous matrices.

#### Higuchi Model

The first example of a mathematical model aimed to describe drug release from a matrix system was proposed by Higuchi in 1961. Initially conceived for planar systems, it was then extended to different geometrics and porous systems.

This model is based on the hypotheses that (i) initial drug concentration in the matrix is much higher than drug solubility; (ii) drug diffusion takes place only in one dimension (edge effect must be negligible); (iii) drug particles are much smaller than system thickness;

 

(iv) matrix swelling and dissolution are negligible; (v) drug diffusivity is constant; and (vi) perfect sink conditions are always attained in the release environment. Accordingly, model expression is given by the equation:

$$Ft = Q = {}_{AD}\sqrt{(2C - Cs)\ Cst}$$

Where Q is the amount of drug released in time t per unit area A, C is the drug initial concentration, Cs is the drug solubility in the matrix media and D is the diffusivity of the drug molecules (diffusion coefficient) in the matrix substance. To study the dissolution from a planar heterogeneous matrix system, where the drug concentration in the matrix is lower than its solubility and the release occurs through pores in the matrix, the expression is given by equation.

$$ft = Q = \frac{D\delta}{\tau}\ 2C - \delta Cs\ Cst$$

where, D is the diffusion coefficient of the drug molecule in the solvent, δ is the porosity of the matrix, $\tau$ is the tortuisity of the matrix and Q, A, Cs and t have the meaning assigned above. Tortuisity is defined as the dimensions of radius and branching of the pores and canals in the matrix. In a general way it is possible to simplify the Higuchi model as (generally known as the simplified Higuchi model):

$$ft = Q = K_H\ .t^{1/2}$$

Where, $K_H$ is the Higuchi dissolution constant.

The data obtained were plotted as cumulative percentage drug release versus square root of time.

Application: This relationship can be used to describe the drug dissolution from several types of modified release pharmaceutical dosage forms, as in the case of some transdermal systems and matrix tablets with water soluble drugs.

**Hixson-Crowell model**

Hixson and Crowell (1931) recognized that the particles regular area is proportional to the cube root of its volume. They derived the equation:

$$W_0^{1/3} - Wt^{1/3} = K_s t$$

Where, $W_0$ is the initial amount of drug in the pharmaceutical dosage form, $W_t$ is the remaining amount of drug in the pharmaceutical dosage form at time t and k (kappa) is a constant incorporating the surface volume relation. The equation describes the release from systems where there is a change in surface area and diameter of particles or tablets.

To study the release kinetics, data obtained from in vitro drug release studies were plotted as cube root of drug percentage remaining in matrix versus time.

Application: this expression applies to pharmaceutical dosage form such as tablets, where the dissolution occurs in planes that are parallel to the drug surface if the tablet dimensions diminish proportionally, in such a manner that the initial geometrical form keeps constant all the time.

The regression analysis of the experimental data was done using statistical functions of the MS-EXCEL program.

## RESULTS AND DISCUSSION

**Standard curve of Levofloxacin**: The $\lambda_{max}$ of Levofloxacin in 0.1N Hydrochloric acid at 293nm as shown in Figure 1. Considering this $\lambda_{max}$, a calibration curve of Levofloxacin in the Figure 2 was frabricated using the data obtained Table 2. The standard graph constructed conferred that the concentrations of drug ranging from 2 to 8 µg/ml obeyed the Beer- Lambert principle. Moreover, the calibration curve of Levofloxacin exihibited a good correlation between the concentrations and absorbance in this range ($R^2$= 0.999).

**Table 2: Data for standard curve of Levofloxacin in 0.1N Hydrochloric acid at 293 nm**

| Concentration (µg/ml) | Absorbance AM* ± S.D |
|---|---|
| 0 | 0.0±0.00 |
| 2 | 0.220±0.016 |
| 4 | 0.459±0.011 |
| 6 | 0.687±0.028 |
| 8 | 0.915±0.027 |
| 10 | 1.33±0.024 |

*****= *Each value is average of three determinations*

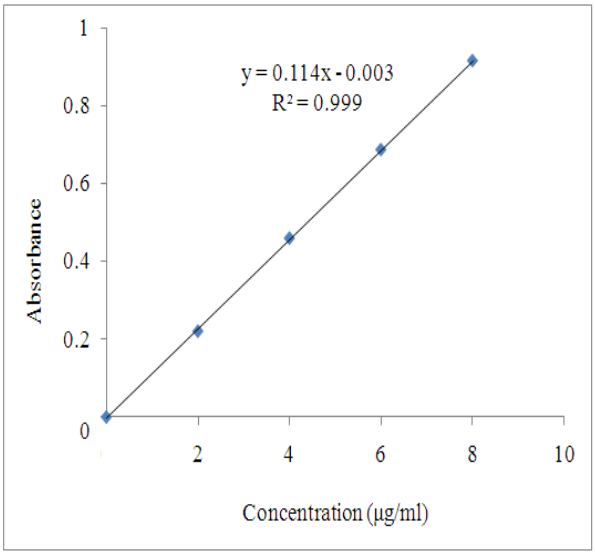


**Figure 2: Standard curve of Levofloxacin in 0.1N Hydrochloric acid at 293nm**

## Characterization of Levofloxacin

### Melting point [7]

The melting point of the Levofloxacin was found to be 212˚C. In Literature, melting point of Levofloxacin was reported to be 214- 216°C. This facilitated the identification of Levofloxacin. The result of melting point determination was reported in Table 3.

 

**Table 3: Melting point of Levofloxacin**

| Melting point of Levofloxacin | Literature value[7] | Observed value |
|---|---|---|
| | 214- 216˚C | 212˚C |

## FT-IR studies[7]

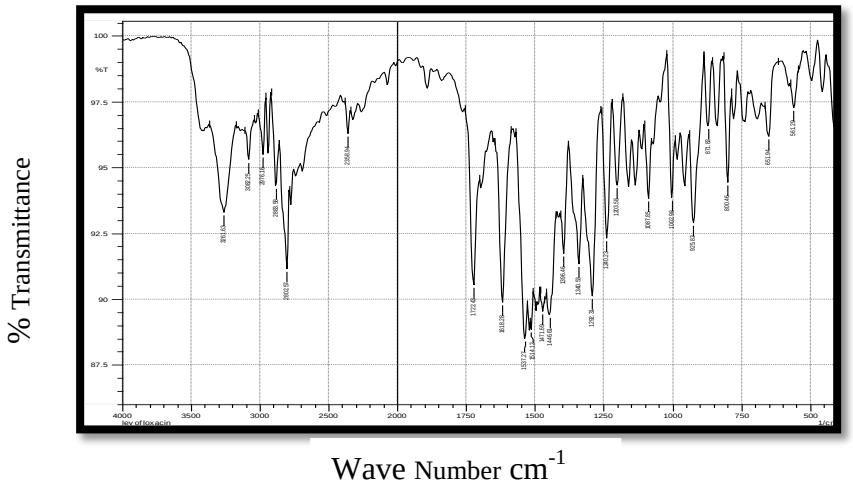


**Figure 3: FT- IR Spectra of Levofloxacin**

The observed characteristic absorption peaks of Levofloxacin were compared with the literature data shown in Table 4. There are three characterization peaks at 1722.43cm$^{-1}$ of carbonyl C=O, 2881.65cm$^{-1}$ of aromatic C-H and 3261.63cm$^{-1}$. The Figure 3 indicating the characteristic peaks of the drug Levofloxacin match with that of the literature data.

**Table 4: Comparison of characteristic IR bands between literature and observed values of Levofloxacin pure drug**

| Characteristic bands | Literature values[7], cm$^{-1}$ | Observed values, cm$^{-1}$ |
|---|---|---|
| Carboxyl, C=O | 1724.81 cm$^{-1}$ | 1722.43 cm$^{-1}$ |
| Aromatic, C-H | 2935.62 cm$^{-1}$ | 2881.65 cm$^{-1}$ |
| O-H group of carboxyl (-COOH) moiety | 3265.81 cm$^{-1}$ | 3261.63 cm$^{-1}$ |

## Drug-Excipient Compatibiliy Studies by FT- IR Studies[7]

Table 4 indicated the three characterization peaks for Levofloxacin reported at 1722.43cm$^{-1}$ of carbonyl C=O, at 2883.58cm$^{-1}$ of aromatic C-H and at 3261.81cm$^{-1}$ of carbonyl –COOH moiety . The comparision of characteristic peaks in the spectra of drug (Table 4 and Figure 3) with the characteristic peaks in the spectra of polymers Gelatin and Acrypol 934P (Table 5 and Figure 4) reveals no drug excipients interaction.

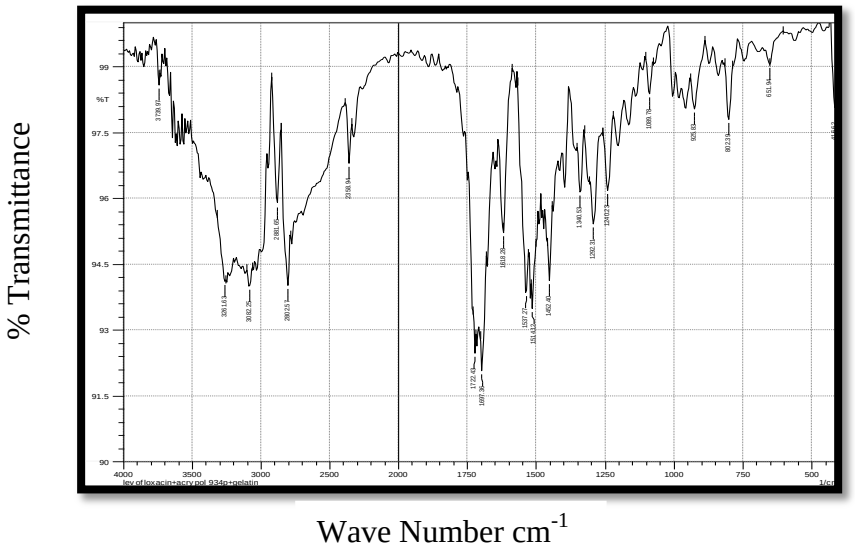


**Figure 4: FT- IR Spectrum of physical mixture Levofloxacin+ Gelatin+ Acrypol 934P**

 

**Table 5: Compatibility study of Levofloxacin with Gelatin & Acrypol 934P**

| Characteristic Bands $cm^{-1}$ | Levofloxacin Observed values $cm^{-1}$ | Physical mixture of Levofloxacin+ Gelatin+ Acrypol 934P Observed values $cm^{-1}$ |
|---|---|---|
| **Carboxyl, C=O** | 1722.43 $cm^{-1}$ | 1722.43 $cm^{-1}$ |
| **Aromatic, C-H** | 2883.58$cm^{-1}$ | 2881.65 $cm^{-1}$ |
| **O-H group of carboxyl (-COOH) moiety** | 3261.63 $cm^{-1}$ | 3261.63 $cm^{-1}$ |

## Gelatin/ Acrypol Mucoadhesive Microspheres

Mucoadhesive Gelatin/ Acrypol 934P microspheres were prepared by Emulsification cross linking method. Hardening of microspheres was performed by chemical cross-linking with Glutaraldehyde.

### Optimization of Process Variables of Gelatin/Acrypol Microspheres

***Effect of Acrypol 934P concentration***

**Table 6: Effect of Acrypol 934P on average particle size of Gelatin / Acrypol 934P microspheres**

| Formulation (Acrypol 934P concentration) | Mean diameter (µm) | Frequency (n) | Average particle size (µm) |
|---|---|---|---|
| **$GA_1$ 0.5%** | 43<br>79<br>115<br>151 | 23<br>52<br>25<br>0 | 70.6µm |
| **$GA_2$ 1%** | 43<br>79<br>115<br>151 | 32<br>55<br>7<br>6 | 81µm |
| **$GA_3$ 2%** | 43<br>79<br>115<br>151 | 18<br>43<br>29<br>10 | 101.6µm |

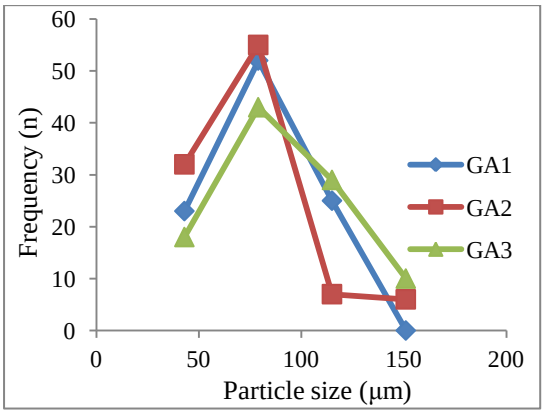


**Figure 5: Plot for effect of Acrypol 934P concentration (with respect to Gelatin mass) on Gelatin / Acrypol 934P microspheres**

The Gelatin/Acrypol microspheres were prepared using varying concentrations of Acrypol 934P (0.5% to 2% with respect to gelatin concentration). The effect of Acrypol 934P concentration on average particle size was observed. The data was tabulated in Table 6.

The particle size for Gelatin/Acrypol 934P microspheres was found to be in the range of 35 µm to 101.6 µm. A direct relationship was observed between polymer concentration and particle size. Particle size was maximum for formulation GA3 (with Acrypol 934P concentration 2%) whereas found to be least for formulation GA1 (with Acrypol 934P concentration 0.5%). Acrypol concentration did not show any remarkable effect on size distribution. Rather it showed a significant effect on bioadhesive property and *in vitro* drug release.

### Effect of stirring speed

The effect of varying stirring speed (500 RPM to 1500 RPM) on the particle size and size distribution of Gelatin / Acrypol 934P microspheres was observed and was reported in the Table 7. There was an inverse relationship found between the stirring speed and average particle size. The mean diameter of microspheres decreased from 81 to 35µm with increasing stirring speed from 500 to 1500 RPM as reported in the Table 7 and Figure 6. Results revealed that the average diameter of microspheres was controlled by rotational speed. The smallest particle size was observed with 1500 RPM but the prepared microspheres adhered to the blades of stirrer and lumps were formed. Hence optimized stirring speed was considered as 1000 RPM although the average particle size was least with 1500 RPM.

 

**Table 7: Effect of stirring speed on Gelatin / Acrypol 934P microspheres**

| Formulation (Stirring speed and Gluteraldehyde concentration) | Mean diameter (µm) | Frequency(n) | Average particle size (µm) |
|---|---|---|---|
| **$GA_2$ 500 RPM 1%** | 50<br>60<br>105<br>150 | 0<br>32<br>55<br>13 | 81µm |
| **$GA_4$ 1000 RPM 1%** | 50<br>60<br>105<br>150 | 25<br>49<br>26<br>0 | 41.5µm |
| **$GA_5$ 1500 RPM 1%** | 50<br>60<br>105<br>150 | 10<br>90<br>0<br>0 | 35µm |

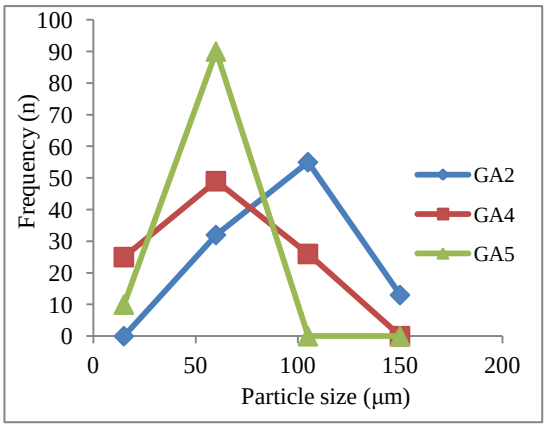


**Figure 6: Plot showing effect of stirring speed on Gelatin / Acrypol 934P microspheres**

## Preparation of Gelatin/Acrypol 934p Mucoadhesive Microspheres

Gelatin/Acrypol microspheres were successfully prepared by emulsification cross linking method. The microspheres produced were spherical. Finally optimized formula was reported in Table 8.

The effect of various process variables such as stirring speed and polymer concentration were optimized. The Gelatin/ Acrypol microspheres were prepared using varying concentrations of Acrypol 934P (0.5% to 2% with respect to gelatin concentration). The particle size for Gelatin/ Acrypol 934P microspheres was increased with higher concentrations of Acrypol polymer and found to be in the range of 70.6 µm to 101.6 µm. Acrypol concentrations did not show any remarkable effect on size distribution.

The mean diameter of microspheres decreased from 101 to 35µm with increasing stirring speed from 500 to 1500 RPM. Results suggested that the stirring speed of 1000 RPM was found to be optimum for Gelatin/ Acrypol 934P microspheres.

**Table 8: Finally optimized formulation [GA4] for Gelatin/ Acrypol 934P microspheres**

| Formulation variables | Parameters |
|---|---|
| **Gelatin solution (%)** | 20 % |
| **Sun flower oil (ml)** | 100 ml |
| **Acrypol 934P (%)** | 1 % |
| **Stirring speed (RPM)** | 1000 RPM |
| **Glutaraldehyde (%)** | 1 % |
| **Acetone (ml)** | 75 ml |
| **Diethyl ether (ml)** | 50 ml |
| **Drug (mg)** | 1000 mg |

## Evaluation Tests of Gelatin/Acrypol Microspheres

### Particle Shape and surface morphology

**Optical microscopy**

Microspheres suspension in liquid paraffin was mounted on glass slide and observed under the optical microscope for their shape. The optical photomicrograph of Gelatin/ Acrypol microspheres was shown in the Figure 7. Photomicrograph suggested the spherical structure with a smooth surface.

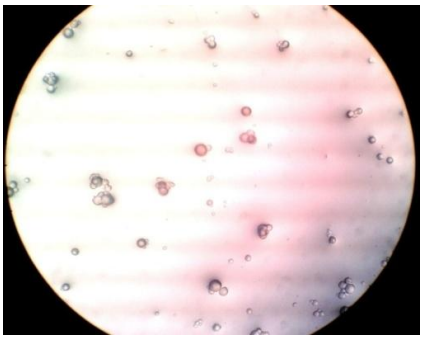

**Figure 7: Optical photomicrograph of Gelatin/ Acrypol microspheres**

 

**SEM analysis**

The surface morphology was visualized by Scaning Electron Microscopy. SEM analysis photograph of Gelatin/ Acrypol microspheres was shown in the Figure 8.

Shape and morphology of the particles was observed in SEM photographs. Figure 8 shows the spherical structure with a smooth surface of Gelatin/ Acrypol 934 P microspheres (Formulation GA4). The optimized formulation GA4 was showed average particle size of 41.5 µm.

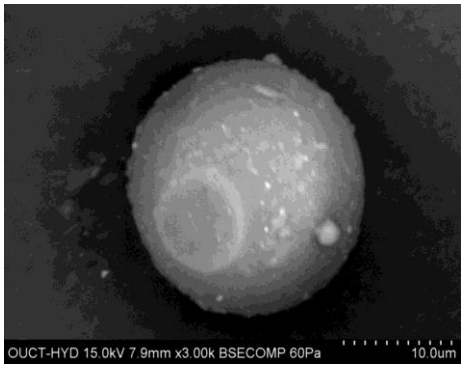


**Figure 8: SEM analysis photograph of Gelatin/ Acrypol microspheres (Formulation GA4)**

**Average particle size**

**Table 9: Average particle size of Gelatin/ Acrypol microspheres**

| Formulation Code | Polymer concentration (%) | Glutaraldehyde concentration (%) | Stirring speed (RPM) | Average particle size (µm) (AM*± S.D ) |
|---|---|---|---|---|
| **GA1** | 0.5% | 1% | 500RPM | 70.6±0.065µm |
| **GA2** | 1% | 1% | 500RPM | 81±0.026µm |
| **GA3** | 2% | 1% | 500RPM | 101.6±0.35µm |
| **GA4** | 1% | 1% | 1000RPM | 41.5±0.054µm |
| **GA5** | 1% | 1% | 1500RPM | 35±0.057µm |

***= Each value is average of three determinations***

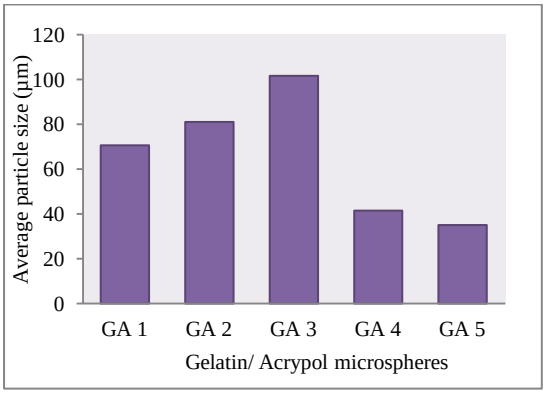


**Figure 9: Average particle size of Gelatin/ Acrypol microspheres formulations**

The size and size distribution of microspheres were determined by optical microscopy. The average particle size for Gelatin/ Acrypol 934P microspheres was tabulated in the Table 9 and reported as a bar diagram in Figure 9. For formulations GA1, GA2 and GA3 the Glutaraldehyde concentration (1%) and stirring speed (500 RPM) were constant with varying polymer concentration. No much difference was seen in average particle size for the above three formulations (GA1, GA2 & GA3) suggesting that there is no direct effect of polymer concentration on particle size of microspheres.

For formulations GA2, GA4 and GA5 the polymer concentration (1%) and Glutaraldehyde concentration (1%) were constant with varying stirring speed from 500 to 1500 RPM. There was an inverse relationship found between the stirring speed and average particle size. The formulation GA5 with highest stirring speed 1500 RPM had smallest particle size of 35µm.

The average particle size (µm) of microspheres decreased (Figure 9) from 101 to 35µm with increasing stirring speed from 500 to 1500 RPM.

**Percentage yield and Drug entrapment efficiency for Gelatin/ Acrypol microspheres**

The percentage yield and drug entrapment efficiency for Gelatin/ Acrypol microspheres was tabulated in the Table 10 and represented as a bar diagram in Figure 10.

The percentage yield for all five Gelatin/ Acrypol 934P microspheres formulation was found in the range of 91.5 to 95.8%. The maximum percentage yield was found with formulation GA4 (94.6%).

The percentage drug entrapment efficiency of the Gelatin/ Acrypol 934P microspheres was represented in Figure 11.

Various drug loaded formulations were prepared by using optimized parameters and studied for percentage drug entrapment efficiency. The results indicating that as the Acrypol 934P concentration increased from 0.5 to 2% the percentage drug entrapment efficiency was increased from 69.25 to 85.43%. The maximum percentage drug entrapment efficiency was shown for (GA4) formulation i.e., 85.43% with Acrypol 934P concentration of 1%.

 

**Table 10: Percentage yield and Drug entrapment efficiency for Gelatin/ Acrypol microspheres**

| Formulation code | Percentage yield | Percentage drug entrapment efficiency (AM*± S.D ) |
|---|---|---|
| **GA1** | 95.8% | 69.25±0.087% |
| **GA2** | 93.6% | 73.46±0.058% |
| **GA3** | 91.5% | 80.02±0.054% |
| **GA4** | 94.6% | 85.43±0.036% |
| **GA5** | 93.4% | 71.8±0.45% |

***= *Each value is average of three determinations*

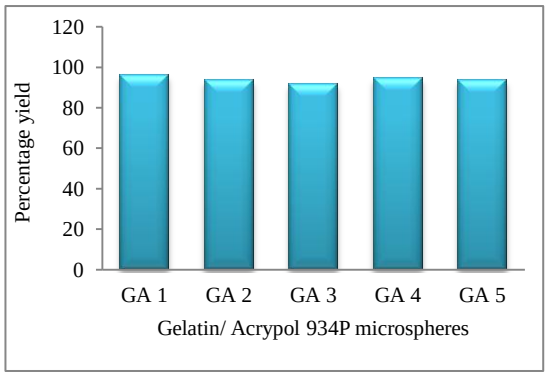


**Figure 10: Chart for percentage yield of Gelatin/ Acrypol 934P microspheres formulations**

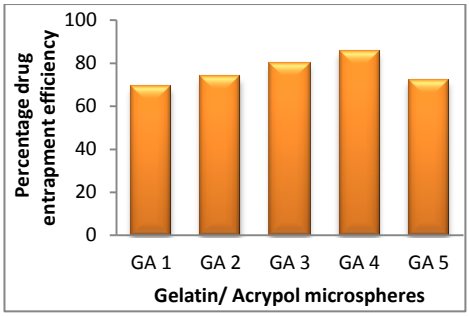


**Figure 11: Percentage Drug entrapment efficiency of Gelatin/ Acrypol microspheres formulations**

### *In- vitro* wash off test

**Table 11: Percentage mucoadhesivity of Gelatin/Acrypol microspheres formulations**

| Formulation code | Percentage mucoadhesivity |
|---|---|
| **GA1** | 81% |
| **GA2** | 85.4% |
| **GA3** | 90.8% |
| **GA4** | 86.6% |
| **GA5** | 87.5% |

The percentage mucoadhesivity of Gelatin/Acrypol microspheres formulations was reported in the Table 11.

The percentage mucoadhesivity for Gelatin/Acrypol microspheres formulations was shown in the Figure 12.

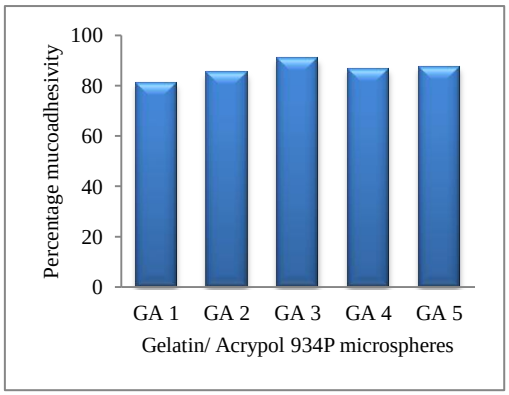


**Figure 12: Percentage mucoadhesivity for Gelatin/ Acrypol microspheres formulations**

The Gelatin/ Acrypol 934P microspheres were prepared by using Gelatin and Acrypol 934P using different concentrations of Acrypol with an intention to increase the mucoadhesion. The percentage mucoadhesivity of Gelatin/ Acrypol 934P microspheres were found to be increased from 81% to 90.8% as the concentration of Acrypol was increased from 0.5% to 2%. There was a forward relationship was found between Acrypol 934P and percentage mucoadhesivity. The highest percentage mucoadhesivity was recorded in GA3 i.e., 90.8% among all formulations because highest concentration of Acrypol 934P i.e., 2% was used in GA3 formulation. Optimized formulation GA4 was showing 86.6% mucoadhesivity. The results clearly showed Table 11 and Figure 12 that surface modification of Gelatin/ Acrypol 934P microspheres greatly enhanced the mucoadhesive property.

When Acrypol 934P was blended with Gelatin, the bioadhesive properties of Acrypol 934P also contributed to overall mucoadhesive capacity of the system (Gelatin- Acrypol 934P microspheres). The carbonyl group of Acrypol 934P provides site for H-bond interaction with sialic acid –COOH group in the mucin glycoprotein. Thus blending of Acrypol 934P with gelatin greatly enhanced the mucoadhesive property.

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### *In vitro* percentage drug release profile of Gelatin/ Acrypol microspheres

**Table 12: *In vitro* percentage drug release profile of Gelatin/ Acrypol microspheres and pure drug (Levofloxacin) in 0.1N Hydrochloric acid.**

| Time (hrs) | Cumulative percentage drug release (AM*± S.D ) | | | | | |
|---|---|---|---|---|---|---|
| | **Pure drug** | **GA1** | **GA2** | **GA3** | **GA4** | **GA5** |
| **1 hr** | 73.2±1.26 | 25.8±0.056 | 19.7±0.59 | 21.4±0.97 | 21±0.28 | 20.3±0.94 |
| **2 hr** | 90.7±0.55 | 39.4±0.54 | 23.9±0.064 | 28.3±0.67 | 25.6±0.38 | 24.5±0.61 |
| **3 hr** | 100±0.07 | 46.9±0.83 | 37.6±0.46 | 37.6±0.67 | 43.3±0.25 | 34.5±0.46 |
| **4 hr** | - | 55.4±0.52 | 45.5±0.13 | 48.7±0.76 | 53.5±0.12 | 39.9±0.45 |
| **5 hr** | - | 61.5±0.58 | 51.3±0.48 | 55.7±0.45 | 61.7±0.47 | 45.6±0.73 |
| **6 hr** | - | 67.8±0.27 | 57±0.82 | 66.1±0.45 | 67.2±0.92 | 65.3±0.42 |
| **7 hr** | - | 71.5±0.57 | 64.9±0.54 | 74±0.82 | 75.2±0.47 | 65.3±0.42 |
| **8 hr** | - | 80.1±0.42 | 70.9±0.57 | 84.5±0.67 | 85.3±0.44 | 76.4±0.33 |

***= Each value is average of three determinations*

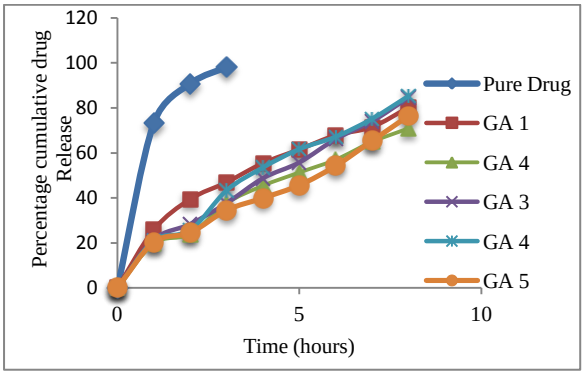


**Figure 13: Plot for *In vitro* cumulative percentage drug release of Gelatin/ Acrypol microspheres in 0.1N Hydrochloric acid.**

The *in vitro* dissolution study was carried out in USP dissolution apparatus by paddle method. Since the stomach pH is between 1 and 3, an acidic medium 0.1N Hydrochloric acid (1.2 pH) was used for dissolution studies. The *in vitro* percentage drug release profile of Gelatin/ Acrypol microspheres and pure drug (Levofloxacin) in 0.1N Hydrochloric acid was tabulated in the Table 12 and represented in Figure 13.

The pure drug release was found to be 73.8% in first hour of dissolution test and complete drug release of total content of capsule was within 3 hours. The release of Levofloxacin from the microspheres follows diffusion or erosion mechanisms through matrix. Thus as long as there is sufficient drug solubility, these mechanism control drug release. A perusal of Figure 13 indicated slow release of Levofloxacin from the formulations.

The influence of concentration of Acrypol 934P on the release from Gelatin/ Acrypol 934P microspheres indicating that drug release increased with increasing Acrypol concentration from 0.5 to 2%, because the amount of swelling was greater for microspheres with higher Acrypol content and the swelling is the principle factor for drug release. The *in vitro* release profile of different microspheres formulations are shown in Figure 13. It was observed that from microspheres formulation sustained drug release was achieved when compared with the pure drug.

### Drug Release Kinetics

The release kinetics of the various formulations was determined to understand the order of drug release. The drug release kinetics of the formulations was determined in 0.1N hydrochloric acid solution and the results are shown in the Table 13 and Figure 14. The release kinetic data indicated that all formulations showed zero order release kinetics (high $R^2$ value for zero order).

The kinetics of drug release from Gelatin/ Acrypol 934P microspheres formulation (GA4) follows zero order (high $R^2$ value) suggesting controlled release of drug.

**Table 13: Release kinetics data of different Gelatin/ Acrypol microspheres formulations.**

| Formulation code | Zero order | | First order | | Higuchi | | Hixon-Crowell | |
|---|---|---|---|---|---|---|---|---|
| | **$K_0$** | **$R^2$** | **$K_1$** | **$R^2$** | **$K_d$** | **$R^2$** | **$K_{hc}$** | **$R^2$** |
| **GA1** | 8.815 | **0.960** | 0.158 | 0.731 | 27.94 | 0.999 | 0.375 | 0.774 |
| **GA2** | 0.133 | **0.930** | 0.00 | 0.00 | 0.00 | 0.00 | 0.380 | 0.818 |
| **GA3** | 9.825 | **0.992** | 0.170 | 0.789 | 29.57 | 0.980 | 0.404 | 0.833 |
| **GA4** | 10.09 | **0.973** | 0.172 | 0.789 | 30.64 | 0.982 | 0.411 | 0.832 |
| **GA5** | 8.518 | **0.988** | 0.164 | 0.789 | 25.48 | 0.970 | 0.382 | 0.829 |

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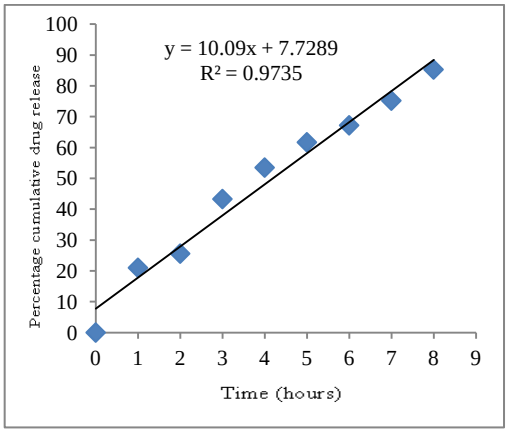


**Figure 14: Plot for zero order kinetics of *in vitro* release of Levofloxacin from Gelatin/ Acrypol 934P microspheres formulation (GA4).**

## CONCLUSION

The conclusions drawn from the present work are drug selected for the present work was Levofloxacin. It was estimated spectrophotometrically scanned at λ max 293 nm. Standard curves for the drug were prepared in 0.1N Hydrochloric acid. The correlation coefficient was found to be 0.999 for standard curve indicating good linearity. The identification was done by IR spectra, which suggested the purity of drug within the prescribed limit. The comparison of spectra of drug with the spectra of polymers Gelatin and Acrypol 934P reveals no drug excipients interaction. The melting point of the Levofloxacin was found to be 212˚C. Gelatin/ Acrypol 934P microspheres of Levofloxacin were prepared by Emulsification cross- linking method. The effect of polymer concentration, Glutaraldehyde concentration and stirring speed was optimized with respect to particle size, size distribution and surface smoothness. It was found that an increase in stirring speed resulted in smaller average particle size and decrease in Glutaraldehyde concentration resulted in better surface smoothness. Results suggested that the stirring speed of 1000RPM was found to be optimum for both types of microspheres. An optimum concentration of Glutaraldehyde (1% with respect to gelatin mass) was a compromise between surface smoothness and *in vitro* digestion of microspheres preparation. Acrypol 934P concentration did not show any remarkable effect on size and size distribution. Rather it shows a significant effect on bioadhesive property and *in vitro* drug release. The percentage yield and drug entrapment efficiency for optimized formulations of Gelatin/ Acrypol microspheres was found to be 94.6%, 85.43%. The average particle size for optimized formulations of Gelatin/ Acrypol 934P microspheres were found to be 41.5µm. Photomicrographs revealed that the microspheres were spherical in shape. *In vitro* drug release was found to be controlled in comparison with pure drug. Drug release from Gelatin/ Acrypol mucoadhesive microspheres showed more than 75% of the drug was released within 8 hrs, while pure drug showed complete drug release within 3 hours. This suggested controlled delivery of Levofloxacin for longer period. Regression analysis revealed that the drug release from the microspheres were followed zero order kinetics. SEM images suggested spherical shape with smooth surface for both type of microspheres formulations. Optimized formulations of Gelatin/ Acrypol 934P microspheres showed excellent mucoadhesivity i.e., 86.5%. Thus, the proposed Gelatin/ Acrypol 934P mucoadhesive microspheres might make a contribution in complete eradication of *Helicobacter pylori* owing to prolonged stomach residence time and small particle size.

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